



Access to PCNA by Srs2 and Elg1 Controls the Choice between Alternative Repair Pathways in *Saccharomyces cerevisiae*

Matan Arbel,^a Alex Bronstein,^a Soumitra Sau,^{a*} Batia Liefshitz,^a Martin Kupiec^a

^aSchool of Molecular Cell Biology and Biotechnology, Tel Aviv University, Ramat Aviv, Israel

Matan Arbel and Alex Bronstein contributed equally. Author order was determined alphabetically.

ABSTRACT During DNA replication, stalling can occur when the replicative DNA polymerases encounter lesions or hard-to replicate regions. Under these circumstances, the processivity factor PCNA gets ubiquitylated at lysine 164, inducing the DNA damage tolerance (DDT) mechanisms that can bypass lesions encountered during DNA replication. PCNA can also be SUMOylated at the same residue or at lysine 127. Surprisingly, *pol30-K164R* mutants display a higher degree of sensitivity to DNA-damaging agents than *pol30-KK127,164RR* strains, unable to modify any of the lysines. Here, we show that in addition to translesion synthesis and strand-transfer DDT mechanisms, an alternative repair mechanism (“salvage recombination”) that copies information from the sister chromatid is repressed by the recruitment of Srs2 to SUMOylated PCNA. Overexpression of Elg1, the PCNA unloader, or of the recombination protein Rad52 allows its activation. We dissect the genetic requirements for this pathway, as well as the interactions between Srs2 and Elg1.

IMPORTANCE PCNA, the ring that encircles DNA maintaining the processivity of DNA polymerases, is modified by ubiquitin and SUMO. Whereas ubiquitin is required for bypassing lesions through the DNA damage tolerance (DDT) pathways, we show here that SUMOylation represses another pathway, salvage recombination. The Srs2 helicase is recruited to SUMOylated PCNA and prevents the salvage pathway from acting. The pathway can be induced by overexpressing the PCNA unloader Elg1, or the homologous recombination protein Rad52. Our results underscore the role of PCNA modifications in controlling the various bypass and DNA repair mechanisms.

KEYWORDS DNA repair, Elg1, Rad52, salvage pathway, Srs2, homologous recombination, yeast

The integrity of the genome is very often compromised by internal and external sources of DNA damage. The vulnerability of the genome increases during DNA replication, when the DNA has to be unpacked and is therefore exposed (1, 2). Chemical modifications of the DNA or proteins bound to it can cause fork stalling or even collapse, leading to a situation in which DNA replication is not completed. To deal with this situation, complex cellular mechanisms have evolved. These response mechanisms act either to promote repair of the lesions or to allow their bypass, thus preventing them from being converted into fatal genomic rearrangements (3). The genetic pathways responsible for DNA repair and genome stability are highly conserved across species. One of these pathways, the DNA damage tolerance (DDT) pathway (also known as the *RAD6* or postreplication repair [PRR] pathway), is activated when single-stranded DNA (ssDNA) accumulates at stalled forks or at gaps created by repriming downstream of the initial stalling lesion (4).

PCNA, the sliding clamp that acts as a processivity factor for replicative DNA

Citation Arbel M, Bronstein A, Sau S, Liefshitz B, Kupiec M. 2020. Access to PCNA by Srs2 and Elg1 controls the choice between alternative repair pathways in *Saccharomyces cerevisiae*. mBio 11:e00705-20. <https://doi.org/10.1128/mBio.00705-20>.

Editor Reed B. Wickner, National Institutes of Health

Copyright © 2020 Arbel et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Martin Kupiec, martin@post.tau.ac.il.

* Present address: Soumitra Sau, Amity Institute of Biotechnology, Amity University Kolkata New Town, Kolkata, West Bengal, India.

This article is a direct contribution from Martin Kupiec, a Fellow of the American Academy of Microbiology, who arranged for and secured reviews by Ayelet Arbel-Eden, Hadassah Academic College, and Shay Covo, Hebrew University of Jerusalem.

Received 24 March 2020

Accepted 8 April 2020

Published 5 May 2020

polymerases, plays an important role in regulating the DDT pathway. To date, two main subpathways have been characterized. The first is activated by monoubiquitylation at lysine 164 of PCNA and is mediated by the E2-conjugating enzyme Rad6 and the E3-ubiquitin ligase Rad18. This ubiquitylation takes place at sites of fork arrest where replication protein A (RPA) accumulates (5, 6) and promotes the exchange of the replicative DNA polymerase by a translesion DNA polymerase able to bypass the DNA lesion by synthesizing DNA in an error-prone manner in most of the cases (7–10) (translesion synthesis [TLS]). Alternatively, the monoubiquitin can be extended by the E2 enzymes Ubc13-Mms2 (UBC13-UEV1 in mammals) together with the E3 Rad5 (or its mammalian orthologs, SHPRH and HLTF) to create K63-linked ubiquitin chains (11). This acts as a signal to direct damage bypass via a template switch (TS) involving the sister chromatid (12, 13). The exact nature of how the polyubiquitylation is recognized and the molecular details of the bypass are still quite mysterious (14), although some form of sequence homology recognition is required (15, 16). In addition to ubiquitylation, PCNA can undergo SUMOylation, predominantly at lysine 164 and, to a lesser extent, at lysine 127 (12). PCNA SUMOylation at K164 seems to be evolutionarily conserved and has been observed also in other organisms (17, 18). SUMOylation of PCNA can lead to the recruitment of Srs2, a UvrD-like helicase that can disrupt Rad51 presynaptic filaments and thus prevent homologous recombination (HR) (19–23). Functional homologs of Srs2 seem to exist in other organisms (e.g., PARI [18] and RTEL1 [24]). PCNA is loaded onto DNA by the Replication Factor C (RFC) complex and is unloaded by an alternative clamp unloader containing the Elg1 protein (25–27). The mammalian ortholog of Elg1, ATAD5, is also a PCNA unloader, important for genome stability, and acts as a tumor-suppressing gene (28).

In addition to TLS and TS, damage can be resolved by a mechanism that involves HR proteins and is independent of PCNA ubiquitylation. This mechanism, here referred to as salvage recombination (SR), is restrained by the Srs2 helicase (14, 29). This pathway is considered a last resort, as unchecked homologous recombination may generate genome instability (30). Although they are sometimes presented as three clear subpathways, the relationship between TLS, TS, and SR is still mysterious, and proteins may be involved in more than one category: for example, Rad5, which is required to initiate the TS subpathway by polyubiquitylating PCNA, also plays a role in the recruitment of TLS polymerases (31). The dissection of the various branches is made even more complex by the variations in timing and location: damage bypass can take place at the fork, or at gaps left behind it by reinitiation; it can also occur in S phase, during the actual replication, or later, in G₂ (4, 32–34).

Here, we analyze the role played by Srs2 in preventing the usage of the SR branch. We show that recruitment of Srs2 by SUMOylation of either K164 or K127 of PCNA abolishes its use. The SR subpathway can, however, be activated by overexpression of Elg1 or Rad52. The SR pathway requires Rad51, Rad52, Rad59, Sgs1, and Elg1 activities.

RESULTS

SRS2 may inhibit DDT by binding to K127 SUMOylated PCNA. To analyze the effect of mutating different genes, we performed quantitative serial dilution assays on a large number of methyl methanesulfonate (MMS) concentrations differing by small increments. This method allows determining the relative sensitivity of all the isogenic strains with high accuracy and consistency.

Mutation of lysine 164 on PCNA prevents this residue from undergoing ubiquitylation or SUMOylation. Lack of these modifications causes sensitivity to DNA-damaging agents, due to the inactivation of the DDT pathways. PCNA can also undergo SUMOylation on lysine 127; however, mutants unable to carry out this modification are not sensitive to DNA-damaging agents. Paradoxically, the double mutant at lysines 127 and 164 (*pol30-KK127,164RR*, here referred to as *pol30-RR*) is less sensitive to DNA damage than the single *K164R* mutant (12) (Fig. 1A). This finding is surprising and suggests that SUMOylation of PCNA at lysine 127 has an inhibitory effect on DNA damage repair or tolerance when K164 is mutated. SUMOylation of PCNA at K164 is coordinated by the

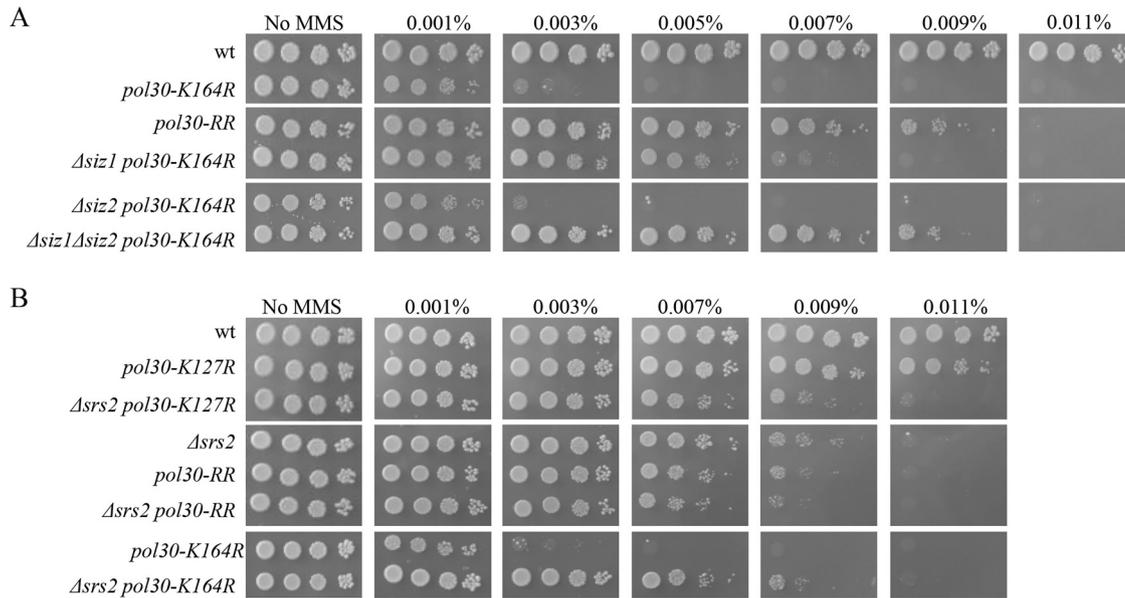


FIG 1 Srs2 and PCNA modifications show epistatic interactions. (A) *Siz1* (and in its absence *Siz2*) has a role in SUMOylation of PCNA on lysine 127. (B) Deletion of *SRS2* exhibits complete epistasis to PCNA modification mutants. Tenfold serial dilutions spotted on plates with increasing concentrations of MMS, photographed after 3 days.

Siz1 E3 ligase (35, 36); however, it is not clear which E3 ligase SUMOylates K127. When we deleted *SIZ1* in the background of *pol30-K164R*, a partial suppression was observed. Since in *Saccharomyces cerevisiae* cells *SIZ2*, a paralog of *SIZ1*, can in some cases compensate for lack of *Siz1* activity and to some extent promote K127 SUMOylation (37), we also combined *pol30-K164R* with Δ *siz2* or Δ *siz1* Δ *siz2*. Although the single Δ *siz2* allele shows no effect, the Δ *siz1* or the Δ *siz1* Δ *siz2* strains showed the same MMS resistance as *pol30-RR* cells (Fig. 1A). Thus, *Siz1* is the main enzyme involved, and inactivation of SUMOylation or mutation of K127 results in a similar effect. The reduced MMS sensitivity of *pol30-K164R* when there is no SUMOylation (Δ *siz1* Δ *siz2* *pol30-K164R* or *pol30-RR*) can be attributed to the lack of recruitment of *Srs2* to PCNA by SUMOylation of lysine K127. Indeed, Fig. 1B shows that mutating lysine 127 in a *pol30-K164R* strain (*pol30-RR*) has the same effect as deleting the *SRS2* gene and that deleting *SRS2* in a *pol30-RR* strain has no further effect. We thus conclude that the recruitment of *Srs2* to PCNA SUMOylated at K127 causes sensitivity to MMS when K164 is unmodified. Deletion of *SRS2* suppresses the high sensitivity to genotoxic agents of Δ *rad18*, Δ *rad5*, and *pol30-K164* mutants, demonstrating that the binding of *Srs2* to SUMOylated K127 has a major role in inhibiting repair (12).

Overexpression of Rad52 and Elg1 suppresses the sensitivity of *pol30-K164R* strains to MMS. The *Srs2* helicase is capable of evicting *Rad51* from DNA and thus inhibiting DNA repair by homologous recombination (HR) (19, 20). It has thus been proposed that *Srs2* bound to PCNA plays a role in preventing uncontrolled recombination events during the S phase. However, the exact mechanism by which *Srs2* sensitizes mutants of the DDT pathway is still unclear.

In a *pol30-K164R* strain, *Srs2* can bind only to K127; yet, the helicase still plays a negative role, as evidenced by the fact that deletion of *SRS2* restores resistance to MMS (Fig. 1B). In order to better understand the mechanism by which *Srs2* exerts its negative effect, we asked whether it was possible to bypass its effect by overexpressing members of the HR machinery.

Figure 2A shows that overexpression (OE) of *Rad52* can partly suppress the sensitivity of *pol30-K164R* to MMS. A weak suppression is also seen by OE of *Rad54* but not by OE of *Rad51*, *Rad55*, *Rad57*, or *Rad59* (Fig. 2A). The fact that *Rad51* had no suppression effect was very surprising, as this recombinase is central to most HR-related

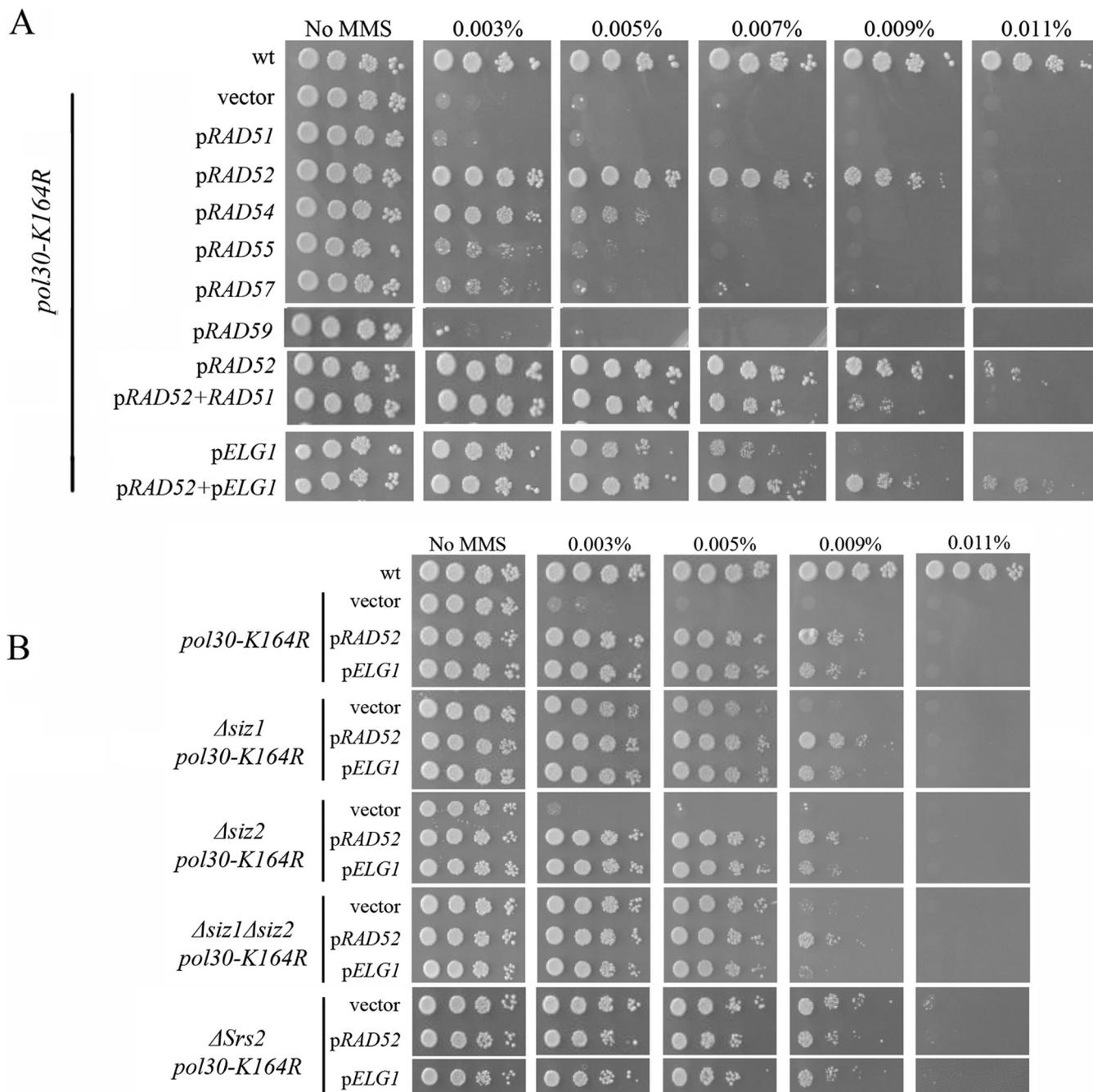


FIG 2 Suppression of MMS sensitivity by overexpression (OE) of Rad52 or Elg1. *pol30-K164R* strains were transformed with various high-copy-number plasmids. (A) OE of Rad52 and Elg1, but not of Rad51 or Rad59, can suppress the sensitivity of *pol30-K164R* to MMS. Elg1 and Rad52 overexpression act in the same pathway. (B) Overexpression of Rad52 or Elg1 is epistatic to Δ *siz1*, Δ *siz2*, Δ *siz1* Δ *siz2*, and Δ *srs2* in the *pol30-K164R* background.

biochemical reactions, and Srs2 is known to inhibit its activity. In addition, cooverexpression of Rad51 and Rad52 gave no further suppression than that provided by Rad52 (Fig. 2A). Therefore, Rad51 protein levels are not limiting for the suppression effect.

In addition to Srs2, Elg1, the unloader of PCNA, also interacts with SUMOylated PCNA (25). By competing for the same binding sites on PCNA, or unloading PCNA altogether, Elg1 may potentially limit the access of Srs2 to the DNA and lower its activity. Overexpression of Elg1 suppresses the MMS sensitivity of *pol30-K164R* almost as well as overexpression of Rad52 (Fig. 2A). We next asked whether *RAD52* and *ELG1* work together or in separate pathways. Overexpressing the two genes together had no additive effect (Fig. 2A), suggesting that they work by a common mechanism.

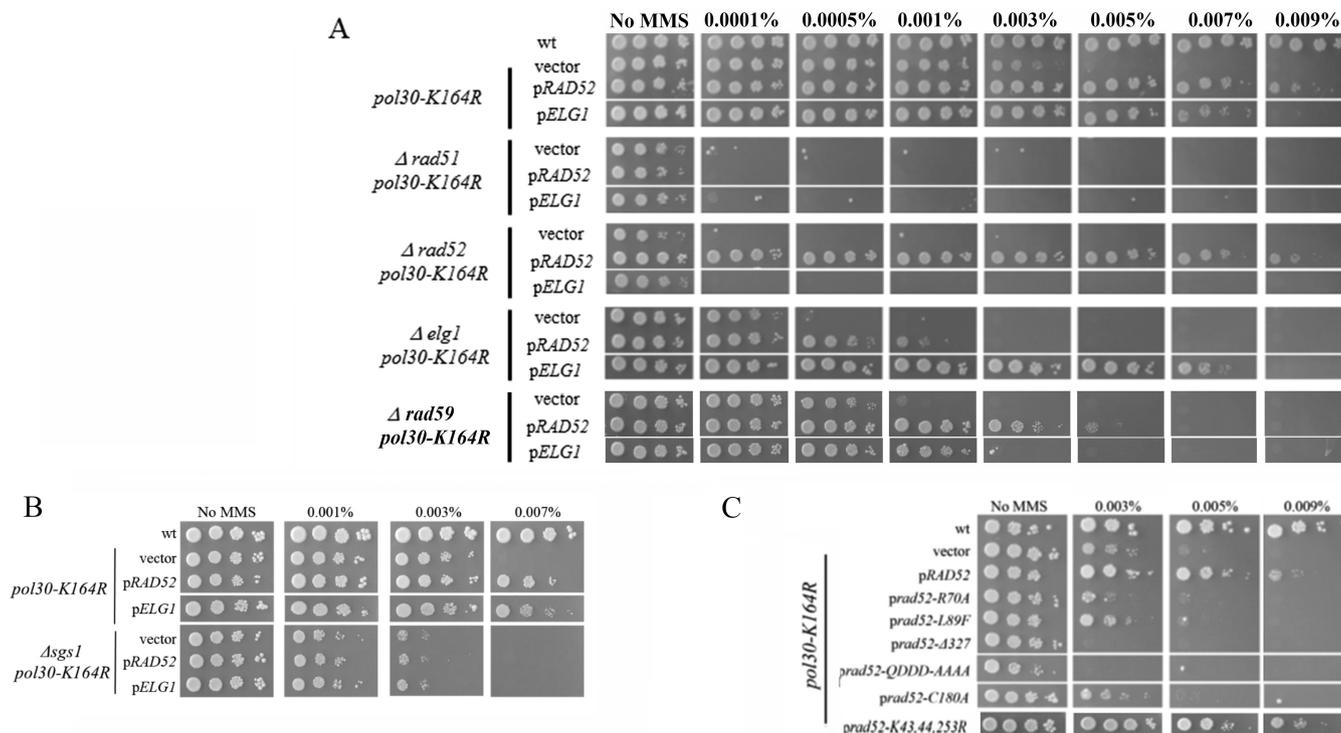


FIG 3 (A) The effect of overexpressing Rad52 and Elg1 on *pol30-K164R* strains requires Rad51, Rad52, Rad59, and Elg1. (B) The effect of overexpressing Rad52 and Elg1 on *pol30-K164R* strains requires Sgs1. (C) Analysis of Rad52's motifs required for the suppression effect. A *pol30-K164R* strain was transformed with high-copy-number plasmids carrying *RAD52* alleles. See text for description of each allele.

Overexpression of Rad52 or of Elg1 allows increased damage tolerance by bypassing Srs2 inhibition. Mutant *pol30-K164R* strains overexpressing Rad52 or Elg1 show a suppression level similar to the one observed in *pol30-K164R* strains carrying the $\Delta siz1 \Delta siz2$ or the $\Delta srs2$ mutations. To test whether different pathways are involved, we overexpressed either Rad52 or Elg1 in *pol30-K164R \Delta siz1 \Delta siz2* (no SUMOylation of PCNA at lysine 127) and *pol30-K164R \Delta srs2* (no Srs2 recruitment) strains. No increased MMS resistance was observed, suggesting that OE of Rad52/Elg1 suppresses *pol30-K164R* sensitivity by acting in the same pathway as that affected by lack of K127 SUMOylation or deletion of Srs2 (Fig. 2B). Interestingly, overexpression of Rad52 or Elg1 resulted in the same level of sensitivity to MMS in *pol30-K164R \Delta siz1*, *pol30-K164R \Delta siz2*, and *pol30-K164R \Delta siz1 \Delta siz2* strains, despite their different sensitivity levels in the absence of the overexpressing plasmids (Fig. 2B). Taken together, these results hint at a common suppression mechanism for Srs2 depletion from the site of DNA damage and for Rad52 or Elg1 overexpression.

To better understand the genetic connection between Rad52 and Elg1, a genetic dependency analysis was carried out. Figure 3A shows that overexpression of either *RAD52* or *ELG1* fails to suppress the *pol30-K164R* sensitivity in the absence of the *RAD51* gene. This suggests that (despite the fact that Rad51 OE had no effect) a Rad51-mediated mechanism is needed for the suppression observed. Moreover, suppression of *pol30-K164R* by *ELG1* OE requires *RAD52*, and suppression by *RAD52* OE is partially dependent on *ELG1*. These results are in line with our previous observation, showing that the two genes work in the same pathway (Fig. 2A). Deletion of *RAD59* partly abolished the suppression of *pol30-K164R* by either *RAD52* or *ELG1* OE. Rad59 is a Rad52 paralog that lacks Rad51-interacting regions and becomes important for DNA repair in the absence of Rad51 (38, 39). Our results show that Rad59 is needed for the efficient work of Rad52 in this pathway, although its protein levels are not limiting, and thus, its overexpression has no effect (Fig. 3A). The salvage recombination pathway is expected to create sister chromatid junctions (SCJs), recombination structures that display ex-

change of strands between sister chromatids. These structures are eventually resolved by the activity of the Sgs1 helicase (40–42). Indeed, deletion of the *SGS1* gene abolished the suppression of *pol30-K164R* by either *RAD52* or *ELG1* OE (Fig. 3B).

In summary, our results suggest a model in which the repair pathway requires the removal of PCNA, a process carried out mainly by Elg1. This in turn allows the Rad52-Rad59 complex to initiate the repair on the damaged site, by annealing to the sister chromatid. Finally, the SCJs thus created are resolved by Sgs1. The partial requirement for Elg1 in the SR pathway (compared to the essentiality of Rad52) can be accounted for by the fact that alternative mechanisms of PCNA unloading exist (and thus, despite its important role during DNA replication, *ELG1* is not an essential gene).

To further analyze the role of Rad52 in the suppression effect, we overexpressed, in the *pol30-K164R* strain, Rad52 mutants defective in specific functions of Rad52 and tested their suppression ability. The *rad52-R70A* and *rad52-L89F* mutants are unable to perform the DNA annealing function of Rad52 (43, 44). Both mutants are unable to suppress *pol30-K164R* (Fig. 3C), implying that the suppression effect acts through the Rad52 DNA annealing ability. The *rad52-Δ327* allele (45) lacks the C-terminal region (C-ter) of Rad52, which is required for interaction with Rad51. The *rad52-QDDD-AAAA* allele (45) is impaired in its interaction with RPA and recombination mediator activity. Both alleles are thus unable to stimulate Rad51 activities. When overexpressed in a *pol30-K164R* strain, they failed to suppress and instead sensitized cells to MMS (Fig. 3C). This again is consistent with a role for Rad51 in the Rad52 OE-stimulated repair of lesions. The *rad52-C180A* allele was described as unable to utilize sister chromatid information for repair (46) and, as expected, also exhibited no suppression effect (Fig. 3C). Rad52 undergoes SUMOylation after DNA damage, and lack of SUMOylation affects its stability but does not affect its recruitment to DNA damage (27, 28). However, Rad52 SUMOylation may also disturb Rad51 filament formation by the activity of the SUMO-targeted Cdc48 segregase, which can limit Rad52-Rad51 physical interaction and displace them from DNA (29, 30). When a *RAD52* allele unable to undergo SUMOylation (*rad52-K43,44,253R*) was overexpressed, it was still as potent a suppressor of the *pol30-K164R* allele (Fig. 3C). We thus conclude that lack of SUMOylation on Rad52 does not affect its suppression capabilities.

The analysis of *rad52* mutants thus suggests that Rad52-mediated sister-chromatid-dependent repair requires the DNA annealing ability of Rad52, as well as its function in promoting assembly of Rad51 nucleofilaments, but not its SUMOylation.

Rad52 and Rad51 interact with the same region of Srs2. Our results until now suggest that in *pol30-K164R* strains, Rad52 and Elg1 OE work in the same SR subpathway, which includes DNA annealing with the sister chromatid and bypasses the restraint exerted by Srs2 recruited to SUMOylated K127 of PCNA. OE of Rad52 may suppress the sensitivity to DNA-damaging agents by facilitating the access of Rad52 to the DNA damage site even when Srs2 is present; Elg1 activity unloads PCNA and thus may reduce the level of Srs2 recruitment and its negative effects. Of note, the inhibition of repair by Srs2 is not likely to be by Rad51 eviction, since OE of Rad51, in contrast to that of Rad52, has no suppression effect. Instead, we assume that Srs2 directly inhibits, or counteracts, the activity of Rad52 (21, 22). In support of this idea, a physical interaction was characterized between Srs2 and Rad52 (47).

We performed yeast two-hybrid (Y2H) experiments in which a plasmid containing Rad52 or Rad51 fused with the transcription-activating domain of Gal4 and a plasmid containing Srs2 fused with the DNA binding domain of Gal4 were both transformed to a histidine auxotroph strain in which the *HIS3* gene has a Gal4-activated promoter. Growth on plates lacking histidine indicates that the two protein have physical interaction. Our own yeast two-hybrid results identified the interaction between Srs2 and Rad52 in a $\Delta rad51$ strain, again implying that this interaction is independent of Rad51 (Fig. 4A). Furthermore, we found that Srs2 binds both Rad51 and Rad52. To pinpoint the exact interaction region of Srs2 and Rad52, we created a set of Y2H constructs carrying various regions of Srs2. Figure 4B shows that a fragment containing amino acids 875 to

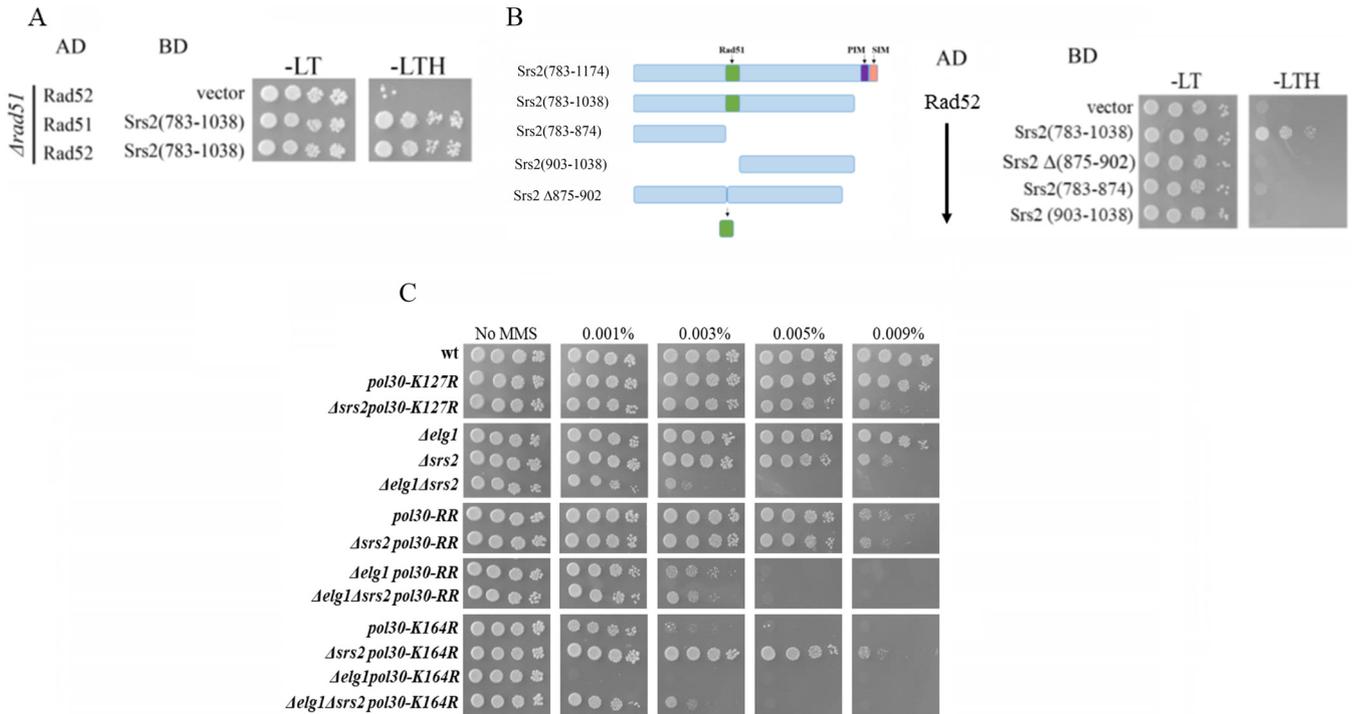


FIG 4 (A) Yeast two-hybrid experiment showing that Rad51 and Rad52 interact with the same region of Srs2, even in the absence of genomic *RAD51*. Plasmids containing either Rad52 or Rad51 fused with the transcription-activating domain of Gal4 and a plasmid containing Srs2 fused with the DNA binding domain of Gal4 were transformed to the yeast strain with leucine and tryptophan selection, respectively. The *HIS3* gene is under a Gal4-dependent promoter and thus was expressed only if physical interactions occurred between the two expressed fusion proteins. –LT, medium lacking leucine and tryptophan; –LTH, medium lacking leucine, tryptophan, and histidine. (B) Yeast two-hybrid experiment showing that Rad52 interacts exclusively with the region of Srs2 between amino acids 875 and 902 and independently of PCNA-interacting and SUMO-interacting motifs (PIM and SIM, respectively). (C) Genetic interactions between $\Delta elg1$, $\Delta srs2$, and PCNA mutants.

902 (the same region that binds Rad51) binds Rad52. In contrast, either the N-terminal region alone, the C-terminal region alone, or the full fragment lacking the Rad51-interacting sequence (875 to 902) fails to show interaction in the Y2H assay (Fig. 4A). Thus, Srs2-Rad51 and Srs2-Rad52 interactions are mediated by the same region of Srs2 and are independent of PCNA- and SUMO-interacting motifs (PIM and SIM, respectively).

The interplay between Srs2 and Elg1 in the SR pathway. What is the relationship between Srs2 and Elg1? The simplest model would imply the following scenario: upon fork stalling, Srs2 is recruited to SUMOylated PCNA, repressing SR. Later, the Elg1 RFC-like complex (RLC) unloads it together with the clamp. This model predicts that $\Delta srs2$ and $\Delta elg1$ should show an epistatic relationship. However, this is not the case: the $\Delta srs2 \Delta elg1$ double mutant is clearly more sensitive than the single mutants (48) (Fig. 4C). How can we explain this phenotype? As expected from our working model, deleting *SRS2* in a *pol30-K164R* strain results in reduced sensitivity to DNA damage, due to the opening of the salvage recombination pathway. Consistent with our results (Fig. 3A), this subpathway relies on PCNA removal from the chromatin, which is done mainly by Elg1. Deleting *ELG1* in the $\Delta srs2 pol30-K164R$ double mutant reduces the sensitivity to the same level seen in the $\Delta elg1 pol30-RR$ (and $\Delta elg1 \Delta srs2 pol30-RR$) mutant: in these strains, the TS and TLS branches are blocked by mutation of lysine 164 and the SR path is partially blocked by lack of Elg1 (Fig. 4C).

Interestingly, the $\Delta elg1 pol30-K164R$ double mutant (in which Srs2 is still active and can be recruited to K127, but the SR subpathway is blocked by the $\Delta elg1$ mutation) shows a level of sensitivity higher than any of the double and triple mutants in which Srs2 recruitment to K127 of PCNA is blocked ($\Delta elg1 \Delta srs2 pol30-K164R$, $\Delta elg1 pol30-RR$, $\Delta elg1 \Delta srs2 pol30-RR$). These results suggest that even in the absence of Elg1, if Srs2 is

not recruited, some repair is carried out. This is consistent with the fact that alternative PCNA-unloading mechanisms exist and that deletion of *ELG1* only partially blocks the SR pathway when Rad52 is overexpressed (Fig. 3A).

Srs2 recruited to SUMOylated K164 has a stronger effect on the SR pathway.

Our results are consistent with a model in which SUMOylation on K127 allows the binding of Srs2, which restricts the activity of Rad52. Next, we compared the recruitment of Srs2 at K127 with its recruitment at K164, the main amino acid of PCNA that undergoes SUMOylation.

First, we validated that $\Delta rad18$ and $\Delta rad5$, responsible for mono- and polyubiquitylation at K164 of PCNA, are not required for the suppression effect of OE Rad52/Elg1. As expected, when Rad52 or Elg1 was overexpressed in a $\Delta rad18$ *pol30-K164R* or a $\Delta rad5$ *pol30-K164R* strain, it still showed suppression (Fig. 5A).

In $\Delta rad18$ or $\Delta rad5$ strains harboring a wild-type (wt) PCNA, the TS and TLS branches in the DDT pathway are inactive, but K164 (in addition to K127) can still be SUMOylated and thus is still able to recruit Srs2. Figure 5B shows that OE of *RAD52* in the background of $\Delta rad18$ or $\Delta rad5$ still causes suppression, albeit weaker than that seen in the *pol30-K164R* background. Interestingly, in these strains, joint OE of Rad52 and Rad51 shows an additive suppression effect that was not seen in *pol30-K164R*. These results imply that Srs2 bound to SUMOylated PCNA at K164 has a stronger negative effect on the SR subpathway, probably by evicting Rad51 from the DNA (19, 20). Overexpression of Rad51, in addition to Rad52, is needed to overcome this negative effect.

In the absence of functional *RAD18* or *RAD5*, when the DTT pathway was inactive, but Srs2 was recruited (mainly to K164), OE of Elg1 completely failed to show any suppressing effect (Fig. 5B), and it had no additive effect with OE of Rad51 (data not shown). We conclude that Srs2 recruited by SUMOylated PCNA at K164 prevents Elg1 from unloading PCNA.

If recruited Srs2 exerts an inhibitory effect on Rad52 and Elg1, then we expect that deletion of *SRS2* in $\Delta rad18/\Delta rad5$ strains will suppress their sensitivity (49, 50), making them as resistant to DNA damage as $\Delta srs2$ alone (as shown for *pol30K164R* [Fig. 1B]). In Fig. 5C, we see that this is the case and that the cells are able to grow in MMS concentrations as high as 0.09% MMS, as do $\Delta srs2$ cells. As expected, the suppression depends on Elg1 activity, and $\Delta rad18/\Delta rad5$ strains deleted for both *SRS2* and *ELG1* become sensitized again to MMS.

DISCUSSION

Recruitment of Srs2 by PCNA SUMOylation at either K127 or K164 controls the SR pathway. The error-free DDT plays a central role in dealing with stalled forks during DNA replication. However, evidence for a Rad18- and Rad5-independent recombinational repair mechanism, which uses information from the sister chromatid to allow repair of damage sites, has been documented and was termed the salvage pathway (15, 16, 51, 52). This mechanism requires Rad51 and Rad52 to invade the sister chromatid DNA and Sgs1 to resolve the joint molecules created by the template switch (14).

The role for Srs2 in regulating the salvage pathway has been deduced from studies showing that deletion of *SRS2*, similarly to $\Delta siz1$, can suppress the DNA damage sensitivity of DDT mutants. The suppression depends on the interaction of Srs2 with SUMOylated PCNA (49, 50). However, the precise interplay between the factors that participate in the DDT and the salvage pathway are not well defined. Moreover, as most SUMOylation events are at K164, the role of K127 SUMOylation has not been described. In this work, we define a role for K127 SUMOylation and characterize the way in which it regulates the SR pathway. By analyzing the suppression level of Srs2, Siz1, and Siz2 in a background of Pol30 mutated in its lysine 164, we found that SUMOylation of lysine 127 is carried out mainly by Siz1. Deletion of *SRS2* suppresses the MMS sensitivity of all the DDT mutants to the same level as *pol30-RR* (Fig. 1B). Although we present results obtained with MMS, similar results were seen with other DNA-damaging agents (data not shown). This suggests that Srs2 has full control over the SR pathway and must be

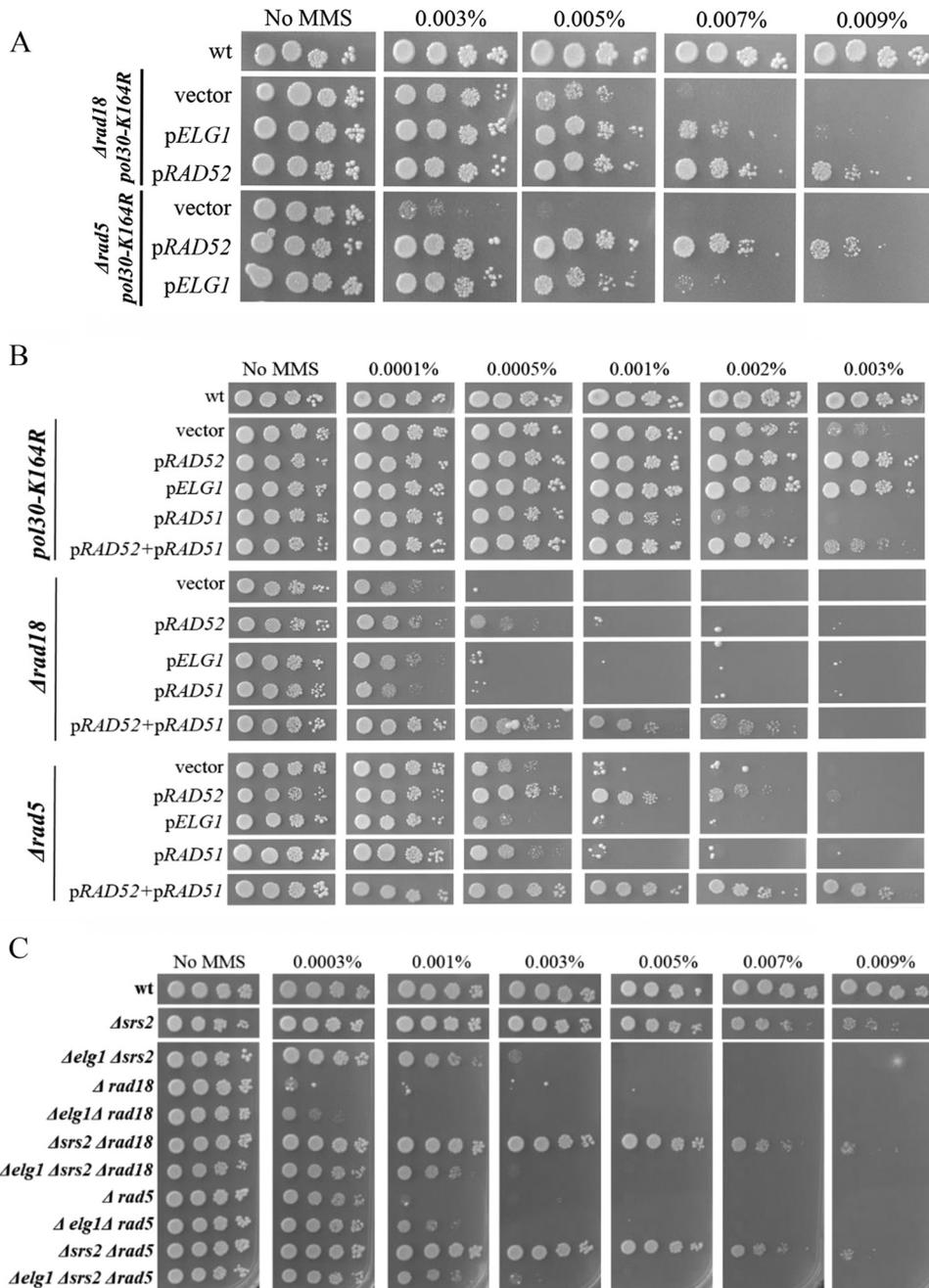


FIG 5 SUMOylation of K164 on PCNA has a role in the regulation of the repair pathways. (A) The suppression effect of *pol30-K164R* by OE of Rad52/Elg1 is still evident when Rad18 or Rad5 is not available. (B) OE of Rad52/Elg1 is less effective when the DDT pathways are inactive, but K164 can bind Srs2. (C) Srs2 deletion suppresses the DDT-defective mutants to the same level.

removed from SUMOylated PCNA as a prerequisite for the activity of the pathway. Following the removal of Srs2, PCNA must be removed (either by Elg1 or by alternative mechanism [25, 27, 53]) to allow repair.

Elg1 and Rad52 promote the initiation of the salvage pathway. OE of Elg1 and Rad52 can suppress the MMS sensitivity of *pol30-K164R* to the same extent as deletion of *SRS2* (Fig. 2B). Further examination revealed that OE of Rad51 has no suppression phenotype by itself, despite the fact that the suppression is dependent on Rad51 (compare Fig. 2A [pRAD51] with Fig. 3A [*Δrad51 pol30-K164R*]). Interestingly, on the background of *Δrad18* or *Δrad5*, Rad51 OE has a phenotype when overexpressed

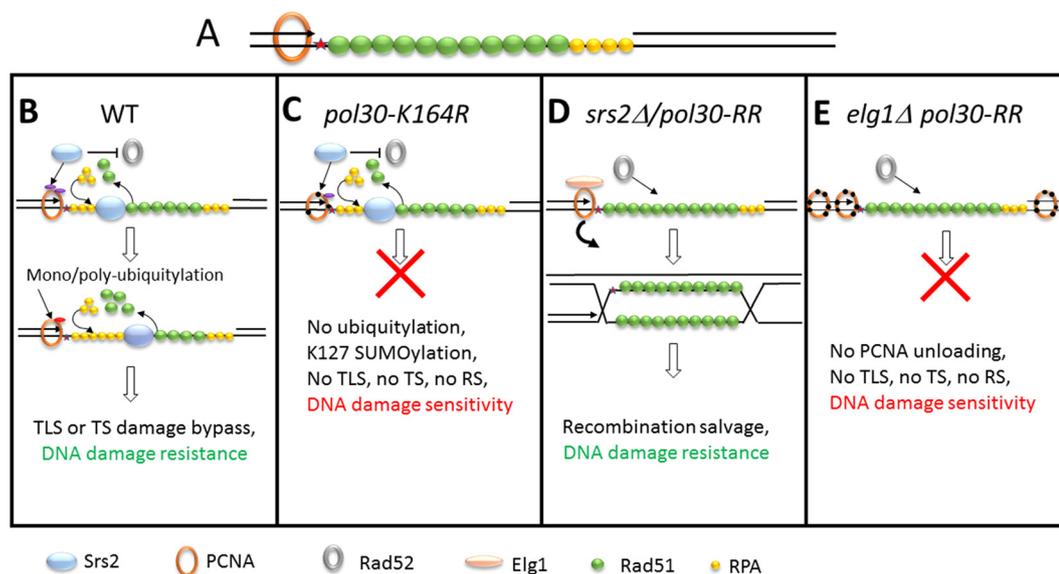


FIG 6 Schematic representation of the recombination salvage pathway regulation. (A) Lesions or perturbations in the DNA (red star) cause fork arrest. The model presented here can take place at the stalled replication fork or, as shown, at ssDNA gaps left behind by arrested replication followed by restart downstream. Events can occur during DNA replication (S phase) or after the bulk of the replication has been completed (G_2 phase). ssDNA will be immediately covered by RPA and Rad51. (B) During normal DNA replication, Srs2 binds to both SUMOylated lysines of PCNA. It inhibits the activity of Rad52 and evicts Rad51, thus precluding homologous recombination events. PCNA ubiquitylation will lead to damage bypass by translesion synthesis (TLS) or by template switch (TS). The choice between these two subpathways may depend on the nature and amount of accumulated DNA damage. (C) When lysine 164 of PCNA is mutated, Srs2 is still recruited by K127 SUMOylation. Srs2 inhibits the activity of Rad52 and Rad51, and since PCNA cannot be ubiquitylated, no DDT subpathway is available. The cells are thus extremely sensitive to DNA damage. (D) When Srs2 is deleted, or both lysines of PCNA that participate in Srs2 recruitment are mutated (*pol30-RR*), the salvage recombination is driven by Rad52 and Rad51. This requires unloading of PCNA by the Elg1 RFC-like complex (although some level of leakage exists). (E) In an *Δelg1 pol30-RR* strain, no TS or TLS recruitment is possible (as PCNA cannot become modified), and Srs2 is not recruited; however, the SR pathway cannot proceed because PCNA cannot be efficiently unloaded. As a consequence, the cells are sensitive to DNA damage.

together with Rad52 (Fig. 5B). This observation, taken together with the fact that Y2H experiments show a direct interaction between Srs2 and Rad52, even in the absence of Rad51 (Fig. 4A and B), shows that Srs2 represses the salvage pathway by directly inhibiting not only Rad51 but also Rad52. Similarly, in the background of *pol30-K164R*, the suppression effect of deleting *SRS2* depends on Elg1 (Fig. 5C), stressing again the important role of Elg1 in SR. We have previously shown that in the absence of Elg1 there is an accumulation of SUMOylated PCNA, and Srs2, and Srs2, on the chromatin (25). Taking all these results into consideration, we propose that Srs2 and Elg1 compete for the interaction with PCNA. Overexpression of Elg1 thus removes the inhibition of Srs2 on SR, either because of direct competition or because higher PCNA-unloading activity by Elg1 leads to lower recruitment of Srs2. As Rad52 is a direct target, it is also possible to overcome the inhibition by overexpressing Rad52. Our results thus clarify the interplay between the various options for repair and lesion bypass. A model explaining the different phenotypes is further elaborated below (Fig. 6).

SUMOylated lysine 164 is the main recruitment site for Srs2. Analysis of the interplay between the two SUMO sites of PCNA when the DDT pathway is not active reveals that deletion of *SRS2* or mutation of lysines 127 and 164 in PCNA suppresses the MMS sensitivity of *Δrad5* or *Δrad18* mutants. This suppression depends on the activity of Elg1 (Fig. 3A and Fig. 5C). Unlike Rad52 OE, Elg1 OE did not show any suppression in *Δrad5* or *Δrad18* cells (Fig. 5B), which are still able to undergo full SUMOylation of PCNA. Furthermore, OE of Rad51 with Rad52 showed additive effects in the background of *Δrad5* or *Δrad18*, but not in *pol30-K164R* (Fig. 2A and Fig. 5B). We interpret these results as follows: SUMOylated lysine 164 is the major recruiter of Srs2. Srs2 bound to SUMOylated lysine 164 regulates the salvage pathway by inhibiting the activities of Rad52 and Rad51. In contrast, recruitment of Srs2 to the SUMOylated lysine 127 of Pol30

TABLE 1 Yeast strain list

Name	Relevant genotype	Reference or source
AB101	MK166 <i>MATa</i>	57
op883	MK166 <i>MATa srs2::KanMX</i>	59
AB270	MK166 <i>MATa pol30-K164R::KanMX srs2::KanMX</i>	22
op710	MK166 <i>MATa elg1::HygMX</i>	Lab stock
op952	MK166 <i>MATa pol30-K164R::LEU2</i>	Lab stock
OP1122	MK166 <i>MATa rad18::LEU2</i>	59
op890	MK166 <i>MATa rad5::KanMX</i>	59
OP1125	MK166 <i>MATa rad18::LEU2 srs2::KanMX</i>	60
AB234	MK166 <i>MATa rad5::KanMX srs2::KanMX</i>	21
MK4137	MK166 <i>MATa sgs1::KanMX</i>	Lab stock
AB89	MK166 <i>MATa pol30-K127R,K164R::KanMX elg1::HygMX</i>	This study
op910	MK166 <i>matα pol30-K127R,K164R::LEU2</i>	Lab stock
op920	MK166 <i>MATa pol30-K127R::LEU2</i>	Lab stock
AB103	MK166 <i>MATa pol30-K164R::KanMX</i>	This study
AB84	MK166 <i>MATa siz1::KanMX pol30-K164R::LEU2</i>	This study
AB144	MK166 <i>MATa pol30-K164R::LEU2 siz12::KanMX siz2::KanMX</i>	This study
AB183	MK166 <i>MATa pol30-K164R::LEU2 siz2::KanMX</i>	This study
AB197	MK166 <i>MATa pol30-K127R::LEU2 srs2::KanMX</i>	This study
AB250	MK166 <i>MATa pol30-K164R::KanMX elg1::HygMX</i>	This study
PJ69-4A	<i>MATa MATa gal4del gal80del GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ</i>	58
KBY 217	<i>pj694 MATα rad51::natNT2</i>	K. A. Bernstein
AB205	MK166 <i>MATa pol30-K164R::LEU2 srs2::KanMX elg1::HygMX</i>	This study
AB312	MK166 <i>MATa pol30-K164R::KanMX rad18::LEU2</i>	This study
AB314	MK166 <i>MATa pol30-K164R::KanMX rad5::KanMX</i>	This study
OP1123	MK166 <i>MATa rad18::LEU2 elg1::HygMX</i>	Lab stock
OP1126	MK166 <i>MATa rad18::LEU2 elg1::HygMX srs2::KanMX</i>	Lab stock
AB249	MK166 <i>MATa rad5::KanMX elg1::HygMX</i>	This study
AB232	MK166 <i>MATa rad5::KanMX elg1::HygMX srs2::KanMX</i>	This study
AB367	MK166 <i>MATa srs2::KanMX elg1::HygMX</i>	22
AB104	MK166 <i>MATa pol30-K164R::KanMX rad51::URA3</i>	This study
AB140	MK166 <i>MATa pol30-K164R::KanMX rad59::KanMX</i>	This study
MK118	MK166 <i>MATa rad59::KanMX</i>	39
AB121	MK166 <i>MATa rad51::LEU2</i>	57
AB124	MK166 <i>MATa rad52::LEU2</i>	57
AB179	MK166 <i>MATa pol30-K164R::KanMX rad52::LEU2</i>	This study

is rarer (SUMOylation on lysine 127 can be detected at a much lower level than on lysine 164). Lower recruitment of Srs2 on the DNA damage site may allow sporadic SR events to take place, explaining the positive OE phenotype of Elg1 in K164R but not in $\Delta rad5$ or $\Delta rad18$. Thus, Srs2 inhibition on lysine 164 of PCNA has a more general role in controlling SR, as opposed to recruitment through lysine 127, which may be time and place specific. Recent experiments have shown, for example, that repair and checkpoint signaling differ at ssDNA gaps and at stalled forks (33). PCNA modifications may be different at these two locations. An alternative, not necessarily exclusive, possibility is that Srs2 bound to K127 acts by inhibiting Rad52, whereas binding to the K164 residue of PCNA activates Srs2's Rad51-evicting activity. Our results could perfectly fit such a scenario.

A model for salvage pathway regulation. Taking all the obtained results together, we propose the following model (Fig. 6). During normal S-phase progression, PCNA at arrested forks or left behind at ssDNA gaps (Fig. 6A) undergoes SUMOylation, and Srs2 can bind to either SUMOylated lysine of PCNA (mainly K164). Srs2 inhibits the activity of Rad52, Rad51, and Elg1, thus preventing untimely/unwanted recombination events. Increased levels of RPA near the arrested PCNA molecule allow it to become monoubiquitylated by the Rad6/Rad18 complex (6) and, if necessary, further polyubiquitylated by the Rad5/Mms2/Ubc13 complex to allow TS (Fig. 6B). The mechanism that decides between these two branches remains mysterious. In *pol30-K164R* strains, Srs2 is still recruited by SUMO at K127, and thus, the SR option is closed, but ubiquitylation of PCNA is precluded by the mutation at K164. This results in increased sensitivity to DNA damage (Fig. 1A and Fig. 6C). In the absence of Srs2, or when PCNA cannot be modified (*pol30-RR*), cells are less sensitive because the SR pathway is open: this requires unloading of PCNA (mainly by Elg1) to invade the sister chromatid, as well as the Rad51, Rad52, Rad59, and Sgs1 proteins (Fig. 6D). Finally,

TABLE 2 Plasmid list

Name	Relevant genotype	Source or reference
WDH262	<i>RAD57</i> in 2 μ m <i>LEU2</i>	David Schild
pYEp13-RAD54	<i>RAD54</i> in 2 μ m <i>LEU2</i>	David Schild
WDH261	<i>RAD52</i> in 2 μ m <i>LEU2</i>	David Schild
AB067B	<i>RAD52</i> in pRS426	Lorraine Symington
WDH264	<i>RAD55</i> in YEp13	Wolf-Dietrich Heyer
WDH265	<i>RAD51</i> in YEp13	Wolf-Dietrich Heyer
pAM21	<i>RAD51</i> in pUC13	Lorraine Symington
AB076B	<i>RAD59</i> in pRS426	Dennis Livingston
opb64b	<i>ELG1</i> N terminus in pGBU9	Lab stock
AB077B	<i>rad52-2</i> in pRS426	Dennis Livingston
AB146	<i>rad52</i> + <i>rad51</i> in pRS426	Dennis Livingston
AB079	<i>rad52-K43,44,253R</i> in pRS426	This study
AB087	<i>rad52-C180A</i> in pRS425	This study
AB088	<i>RAD52</i> in pRS425	This study
MK3561	<i>rad52-QDDD-AAAA</i> in pRS325	45
MK3562	<i>rad52-Δ327</i> in pRS325	45
MK3563	<i>rad52-R70A</i> in pRS325	45
MK3563	<i>rad52-R70A</i> in pRS325	45
MK3564	<i>rad52-L89F</i> in pRS325	45
MK3354	<i>RAD52</i> in pGAD424	Giora Simchen
MK3353	<i>RAD51</i> in pGAD424	Giora Simchen
D2084	<i>srs2(783–1174)</i> in pGBDC2	Stefan Jentsch
D2089	<i>srs2(783–1038)</i> in pGBD2	Stefan Jentsch
AB169	<i>srs2(783–1038)Δ875–902</i> in pGBD2	This study
AB154	<i>srs2(783–874)</i> in pGBT9	This study
AB156	<i>srs2(903–1038)</i> in pGBT9	This study
AB135	<i>SRS2</i> in pRS425	This study
AB133	<i>srs2-K41A</i> in pRS425	This study
AB139	<i>srs2Δ(875–902)</i> in pRS425	This study
p195- <i>ELG1</i>	<i>ELG1-myc7HIS</i> in pYEplac195	Lab stock
MK1969	<i>SMT3-pol30-K127R,K164R</i> in pGBT9	59

deleting *ELG1* in the *pol30-RR* strain leaves the cells with only a partially active SR option and thus more sensitive to DNA damage (Fig. 6E).

Our results shed light on the important role that PCNA and its modifications have in determining the type of lesion bypass/repair used by the cell and show how complex these decisions are. They also better define the role of Srs2 in the repair of damage during DNA replication. In this study, we have used a minimalist approach and left out additional levels of regulation, such as Srs2 modifications and cellular regulators. Additional players that have an impact on pathway choice are Uls1 (54), Esc2 (29), Mph1/Mhf1-2 (55, 56), and others. Future work will center on the role played by these proteins, as well as the choice between TS and TLS, both of which require PCNA ubiquitylation.

MATERIALS AND METHODS

Yeast strains. Unless differently stated, all strains are derivatives of MK166, which has the following genotype: *MATa lys2::Ty1Sup ade2-1(o) can1-100(o) ura3-52 leu2-3,112 his3del200 trp1del901 HIS3::lys2::ura3 his4::TRP1::his4* (57).

Standard yeast molecular genetics techniques were used to delete individual genes.

Strain and plasmid lists are available in Tables 1 and 2, respectively.

DNA damage sensitivity serial dilution assay. Serial 10-fold dilutions of logarithmic yeast cells were spotted on fresh synthetic dextrose (SD)-complete (or SD lacking a specific amino acid to preserve the plasmid) plates with or without different concentrations of methyl methanesulfonate (MMS) (Sigma) and incubated at 30°C for 3 days. MMS plates were freshly prepared, dried in a biological hood, and used the same day.

Chromatin fractionation assay. Fifty milliliters of a logarithmic culture was collected and washed with double-distilled water (ddH₂O), pre-Sorbitol buffer (PSB; 20 mM Tris-HCl, pH 7.4, 2 mM EDTA, 100 mM NaCl, 10 mM β -mercaptoethanol [β -ME]) and Sorbitol Buffer (SB; 1 M sorbitol, 20 mM Tris-HCl, pH 7.4). Next, cells were suspended in 1 ml SB, 30 μ l Zymolyase 20T (20 mg/ml in SB) was added, and samples were incubated at 30°C until spheroplasts were visible (around 1 h). Spheroplasts were washed twice with SB and suspended in 500 ml EBX (20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.25% Triton X-100, 15 mM β -ME plus protease/phosphatase inhibitors). Triton X-100 was added to an 0.5% final concentration to lyse the outer cell membrane, and the samples were kept on ice for 10 min with gentle mixing.

Whole-cell lysate (whole-cell extract [WCE]) samples were taken, and the rest of the lysate was layered over 1 ml NIB (20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1.2 M sucrose, 15 mM β -ME plus protease/phosphatase inhibitors). After centrifugation, the cytoplasmic fraction was taken. The nuclear pellet was suspended in 500 μ l EBX, and Triton X-100 was added to a 1% final concentration to lyse the nuclear membrane. The pellet was centrifuged, and the chromatin was suspended in 50 μ l Tris (pH 8.0) for Western analysis (chromatin). For Western blotting, the following antibodies were used: PCNA antibody (1:1,000; Abcam), histone H3 (1:5,000; Abcam), and RPS6 (1:1,000; Abcam).

Yeast two-hybrid assay. To detect two hybrid interactions, yeast strain PJ69 (58) was cotransformed with a *LEU2*-marked plasmid containing genes fused to the *GAL4* activating domain (pACT or pGAD424) and a plasmid containing genes fused to the *GAL4* DNA binding domain (pGBU9). Yeast cultures were grown in the respective selective media to retain the plasmids, with or without MMS. Cells were incubated for 3 to 5 days at 30°C.

ACKNOWLEDGMENTS

We thank Kara Bernstein, Wolf Heyer, Giora Simchen, Lorraine Symington, Hele Ulrich, Lumir Krejci, and the late Stefan Jentsch for strains and plasmids and past and present members of the Kupiec lab for support, ideas, and encouragement.

This work was supported by grants from the Israel Science Foundation, the Minerva Center, and the Israel Cancer Research Fund to M.K. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. S.S. is currently a Ramalingaswami Fellow, supported by DBT, Government of India.

REFERENCES

- Corcoles-Saez I, Dong K, Cha RS. 2019. Versatility of the Mec1(ATM/ATR) signaling network in mediating resistance to replication, genotoxic, and proteotoxic stresses. *Curr Genet* 65:657–661. <https://doi.org/10.1007/s00294-018-0920-y>.
- Moriel-Carretero M, Pasero P, Pardo B. 2019. DDR Inc., one business, two associates. *Curr Genet* 65:445–451. <https://doi.org/10.1007/s00294-018-0908-7>.
- Wong RP, García-Rodríguez N, Zilio N, Hanulová M, Ulrich HD. 2020. Processing of DNA polymerase-blocking lesions during genome replication is spatially and temporally segregated from replication forks. *Mol Cell* 77:3–16.e4. <https://doi.org/10.1016/j.molcel.2019.09.015>.
- Karras GI, Jentsch S. 2010. The RAD6 DNA damage tolerance pathway operates uncoupled from the replication fork and is functional beyond S phase. *Cell* 141:255–267. <https://doi.org/10.1016/j.cell.2010.02.028>.
- Davies AA, Huttner D, Daigaku Y, Chen S, Ulrich HD. 2008. Activation of ubiquitin-dependent DNA damage bypass is mediated by replication protein A. *Mol Cell* 29:625–636. <https://doi.org/10.1016/j.molcel.2007.12.016>.
- Li S, Dong Z, Yang S, Feng J, Li Q. 2019. Chaperoning RPA during DNA metabolism. *Curr Genet* 65:857–864. <https://doi.org/10.1007/s00294-019-00945-3>.
- Stelter P, Ulrich HD. 2003. Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. *Nature* 425:188–191. <https://doi.org/10.1038/nature01965>.
- Acharya N, Manohar K, Peroumal D, Khandagale P, Patel SK, Sahu SR, Kumari P. 2019. Multifaceted activities of DNA polymerase ϵ : beyond translesion DNA synthesis. *Curr Genet* 65:649–656. <https://doi.org/10.1007/s00294-018-0918-5>.
- Bebenek A, Ziuzia-Graczyk I. 2018. Fidelity of DNA replication—a matter of proofreading. *Curr Genet* 64:985–996. <https://doi.org/10.1007/s00294-018-0820-1>.
- Szwajczak E, Fijalkowska IJ, Suski C. 2018. The importance of an interaction network for proper DNA polymerase zeta heterotetramer activity. *Curr Genet* 64:575–580. <https://doi.org/10.1007/s00294-017-0789-1>.
- Fan Q, Xu X, Zhao X, Wang Q, Xiao W, Guo Y, Fu YV. 2018. Rad5 coordinates translesion DNA synthesis pathway by recognizing specific DNA structures in *Saccharomyces cerevisiae*. *Curr Genet* 64:889–899. <https://doi.org/10.1007/s00294-018-0807-y>.
- Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. 2002. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* 419:135–141. <https://doi.org/10.1038/nature00991>.
- Branzei D, Psakhye I. 2016. DNA damage tolerance. *Curr Opin Cell Biol* 40:137–144. <https://doi.org/10.1016/j.ccb.2016.03.015>.
- Branzei D, Szakal B. 2016. DNA damage tolerance by recombination: molecular pathways and DNA structures. *DNA Repair (Amst)* 44:68–75. <https://doi.org/10.1016/j.dnarep.2016.05.008>.
- Branzei D, Vanoli F, Foiani M. 2008. SUMOylation regulates Rad18-mediated template switch. *Nature* 456:915–920. <https://doi.org/10.1038/nature07587>.
- Zhang H, Lawrence CW. 2005. The error-free component of the RAD6/RAD18 DNA damage tolerance pathway of budding yeast employs sister-strand recombination. *Proc Natl Acad Sci U S A* 102:15954–15959. <https://doi.org/10.1073/pnas.0504586102>.
- Gali H, Juhasz S, Morocz M, Hajdu I, Fatyol K, Szukacsov V, Burkovics P, Haracska L. 2012. Role of SUMO modification of human PCNA at stalled replication fork. *Nucleic Acids Res* 40:6049–6059. <https://doi.org/10.1093/nar/gks256>.
- Moldovan G-L, Dejsuphong D, Petalcorin MIR, Hofmann K, Takeda S, Boulton SJ, D'Andrea AD. 2012. Inhibition of homologous recombination by the PCNA-interacting protein PARI. *Mol Cell* 45:75–86. <https://doi.org/10.1016/j.molcel.2011.11.010>.
- Krejci L, Van Komen S, Li Y, Villemain J, Reddy MS, Klein H, Ellenberger T, Sung P. 2003. DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature* 423:305–309. <https://doi.org/10.1038/nature01577>.
- Veaute X, Jeusset J, Soustelle C, Kowalczykowski SC, Le Cam E, Fabre F. 2003. The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature* 423:309–312. <https://doi.org/10.1038/nature01585>.
- Bronstein A, Bramson S, Shemesh K, Liefshitz B, Kupiec M. 2018. Tight regulation of Srs2 helicase activity is crucial for proper functioning of DNA repair mechanisms. *G3 (Bethesda)* 8:1615–1626. <https://doi.org/10.1534/g3.118.200181>.
- Bronstein A, Gershon L, Grinberg G, Alonso-Perez E, Kupiec M. 2018. The main role of Srs2 in DNA repair depends on its helicase activity, rather than on its interactions with PCNA or Rad51. *mBio* 9:e01192-18. <https://doi.org/10.1128/mBio.01192-18>.
- Piazza A, Heyer WD. 2019. Moving forward one step back at a time: reversibility during homologous recombination. *Curr Genet* 65:1333–1340. <https://doi.org/10.1007/s00294-019-00995-7>.
- Barber LJ, Youds JL, Ward JD, McIlwraith MJ, O'Neil NJ, Petalcorin MIR, Martin JS, Collis SJ, Cantor SB, Auclair M, Tissenbaum H, West SC, Rose AM, Boulton SJ. 2008. RTEL1 maintains genomic stability by suppressing homologous recombination. *Cell* 135:261–271. <https://doi.org/10.1016/j.cell.2008.08.016>.
- Parnas O, Zipin-Roitman A, Pfander B, Liefshitz B, Mazor Y, Ben-Aroya S, Jentsch S, Kupiec M. 2010. Elg1, an alternative subunit of the RFC clamp

- loader, preferentially interacts with SUMOylated PCNA. *EMBO J* 29: 2611–2622. <https://doi.org/10.1038/emboj.2010.128>.
26. Kubota T, Nishimura K, Kanemaki MT, Donaldson AD. 2013. The Elg1 replication factor C-like complex functions in PCNA unloading during DNA replication. *Mol Cell* 50:273–280. <https://doi.org/10.1016/j.molcel.2013.02.012>.
 27. Shemesh K, Sebesta M, Pacesa M, Sau S, Bronstein A, Parnas O, Liefshitz B, Venclovas C, Krejci L, Kupiec M. 2017. A structure-function analysis of the yeast Elg1 protein reveals the importance of PCNA unloading in genome stability maintenance. *Nucleic Acids Res* 45:3189–3203. <https://doi.org/10.1093/nar/gkw1348>.
 28. Bell DW, NISC Comparative Sequencing Program, Sikdar N, Lee KY, Price JC, Chatterjee R, Park HD, Fox J, Ishiai M, Rudd ML, Pollock LM, Fogoros SK, Mohamed H, Hanigan CL, Zhang S, Cruz P, Renaud G, Hansen NF, Cherukuri PF, Borate B, McManus KJ, Stoepel J, Sipahimalani P, Godwin AK, Sgroi DC, Merino MJ, Elliot G, Elkhoulou A, Vinson C, Takata M, Mullikin JC, Wolfsberg TG, Hieter P, Lim DS, Myung K. 2011. Predisposition to cancer caused by genetic and functional defects of mammalian atad5. *PLoS Genet* 7:e1002245. <https://doi.org/10.1371/journal.pgen.1002245>.
 29. Urulagodi M, Sebesta M, Menolfi D, Szakal B, Sollier J, Sisakova A, Krejci L, Branzei D. 2015. Local regulation of the Srs2 helicase by the SUMO-like domain protein Esc2 promotes recombination at sites of stalled replication. *Genes Dev* 29:2067–2080. <https://doi.org/10.1101/gad.265629.115>.
 30. Lambert S, Carr AM. 2013. Replication stress and genome rearrangements: lessons from yeast models. *Curr Opin Genet Dev* 23: 132–139. <https://doi.org/10.1016/j.gde.2012.11.009>.
 31. Gallo D, Kim T, Szakal B, Saayman X, Narula A, Park Y, Branzei D, Zhang Z, Brown GW. 2019. Rad5 recruits error-prone DNA polymerases for mutagenic repair of ssDNA gaps on undamaged templates. *Mol Cell* 73:900–914.e9. <https://doi.org/10.1016/j.molcel.2019.01.001>.
 32. Daigaku Y, Davies AA, Ulrich HD. 2010. Ubiquitin-dependent DNA damage bypass is separable from genome replication. *Nature* 465:951–955. <https://doi.org/10.1038/nature09097>.
 33. Garcia-Rodriguez N, Morawska M, Wong RP, Daigaku Y, Ulrich HD. 2018. Spatial separation between replisome- and template-induced replication stress signaling. *EMBO J* 37:e98369. <https://doi.org/10.15252/emboj.201798369>.
 34. Garcia-Rodriguez N, Wong RP, Ulrich HD. 2018. The helicase Pif1 functions in the template switching pathway of DNA damage bypass. *Nucleic Acids Res* 46:8347–8356. <https://doi.org/10.1093/nar/gky648>.
 35. Halas A, Podlaska A, Derkacz J, McIntyre J, Skoneczna A, Sledziwska-Gojska E. 2011. The roles of PCNA SUMOylation, Mms2-Ubc13 and Rad5 in translesion DNA synthesis in *Saccharomyces cerevisiae*. *Mol Microbiol* 80:786–797. <https://doi.org/10.1111/j.1365-2958.2011.07610.x>.
 36. Burkovics P, Sebesta M, Sisakova A, Plault N, Szukacsov V, Robert T, Pinter L, Marini V, Kolesar P, Haracska L, Gangloff S, Krejci L. 2013. Srs2 mediates PCNA-SUMO-dependent inhibition of DNA repair synthesis. *EMBO J* 32:742–755. <https://doi.org/10.1038/emboj.2013.9>.
 37. Chen XL, Reindle A, Johnson ES. 2005. Misregulation of 2 microm circle copy number in a SUMO pathway mutant. *Mol Cell Biol* 25:4311–4320. <https://doi.org/10.1128/MCB.25.10.4311-4320.2005>.
 38. Bai Y, Symington LS. 1996. A Rad52 homolog is required for RAD51-independent mitotic recombination in *Saccharomyces cerevisiae*. *Genes Dev* 10:2025–2037. <https://doi.org/10.1101/gad.10.16.2025>.
 39. Jablonovich Z, Liefshitz B, Steinlauf R, Kupiec M. 1999. Characterization of the role played by the RAD59 gene of *Saccharomyces cerevisiae* in ectopic recombination. *Curr Genet* 36:13–20. <https://doi.org/10.1007/s002940050467>.
 40. Branzei D, Sollier J, Liberi G, Zhao X, Maeda D, Seki M, Enomoto T, Ohta K, Foiani M. 2006. Ubc9- and mms21-mediated sumoylation counteracts recombinogenic events at damaged replication forks. *Cell* 127:509–522. <https://doi.org/10.1016/j.cell.2006.08.050>.
 41. Chu WK, Hickson ID. 2009. RecQ helicases: multifunctional genome caretakers. *Nat Rev Cancer* 9:644–654. <https://doi.org/10.1038/nrc2682>.
 42. Li F, Ball LG, Fan L, Hanna M, Xiao W. 2018. Sgs1 helicase is required for efficient PCNA monoubiquitination and translesion DNA synthesis in *Saccharomyces cerevisiae*. *Curr Genet* 64:459–468. <https://doi.org/10.1007/s00294-017-0753-0>.
 43. Shi I, Hallwyl SC, Seong C, Mortensen U, Rothstein R, Sung P. 2009. Role of the Rad52 amino-terminal DNA binding activity in DNA strand capture in homologous recombination. *J Biol Chem* 284:33275–33284. <https://doi.org/10.1074/jbc.M109.057752>.
 44. Cortés-Ledesma F, Malagón F, Aguilera A. 2004. A novel yeast mutation, rad52-L89F, causes a specific defect in Rad51-independent recombination that correlates with a reduced ability of Rad52-L89F to interact with Rad59. *Genetics* 168:553–557. <https://doi.org/10.1534/genetics.104.030551>.
 45. Lee M, Lee CH, Demin AA, Munashingha PR, Amangyeld T, Kwon B, Formosa T, Seo YS. 2014. Rad52/Rad59-dependent recombination as a means to rectify faulty Okazaki fragment processing. *J Biol Chem* 289: 15064–15079. <https://doi.org/10.1074/jbc.M114.548388>.
 46. Munoz-Galvan S, Jimeno S, Rothstein R, Aguilera A. 2013. Histone H3K56 acetylation, Rad52, and non-DNA repair factors control double-strand break repair choice with the sister chromatid. *PLoS Genet* 9:e1003237. <https://doi.org/10.1371/journal.pgen.1003237>.
 47. Kolesar P, Altmannova V, Silva S, Lisby M, Krejci L. 2016. Pro-recombination role of Srs2 protein requires SUMO (small ubiquitin-like modifier) but is independent of PCNA (proliferating cell nuclear antigen) interaction. *J Biol Chem* 291:7594–7607. <https://doi.org/10.1074/jbc.M115.685891>.
 48. Gazy I, Liefshitz B, Bronstein A, Parnas O, Atias N, Sharan R, Kupiec M. 2013. A genetic screen for high copy number suppressors of the synthetic lethality between elg1Delta and srs2Delta in yeast. *G3 (Bethesda)* 3:917–926. <https://doi.org/10.1534/g3.113.005561>.
 49. Schiestl RH, Prakash S, Prakash L. 1990. The SRS2 suppressor of rad6 mutations of *Saccharomyces cerevisiae* acts by channeling DNA lesions into the RAD52 DNA repair pathway. *Genetics* 124:817–831.
 50. Pfander B, Moldovan GL, Sacher M, Hoegge C, Jentsch S. 2005. SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. *Nature* 436:428–433. <https://doi.org/10.1038/nature03665>.
 51. Vanoli F, Fumasoni M, Szakal B, Maloisel L, Branzei D. 2010. Replication and recombination factors contributing to recombination-dependent bypass of DNA lesions by template switch. *PLoS Genet* 6:e1001205. <https://doi.org/10.1371/journal.pgen.1001205>.
 52. Minca EC, Kowalski D. 2010. Multiple Rad5 activities mediate sister chromatid recombination to bypass DNA damage at stalled replication forks. *Mol Cell* 38:649–661. <https://doi.org/10.1016/j.molcel.2010.03.020>.
 53. Kubota T, Katou Y, Nakato R, Shirahige K, Donaldson AD. 2015. Replication-coupled PCNA unloading by the Elg1 complex occurs genome-wide and requires Okazaki fragment ligation. *Cell Rep* 12: 774–787. <https://doi.org/10.1016/j.celrep.2015.06.066>.
 54. Kramarz K, Mucha S, Litwin I, Barg-Wojas A, Wysocki R, Dziadkowiec D. 2017. DNA damage tolerance pathway choice through Uls1 modulation of Srs2 SUMOylation in *Saccharomyces cerevisiae*. *Genetics* 206:513–525. <https://doi.org/10.1534/genetics.116.196568>.
 55. Singh S, Shemesh K, Liefshitz B, Kupiec M. 2013. Genetic and physical interactions between the yeast ELG1 gene and orthologs of the Fanconi anemia pathway. *Cell Cycle* 12:1625–1636. <https://doi.org/10.4161/cc.24756>.
 56. Dae DL, Ferrari E, Longerich S, Zheng XF, Xue X, Branzei D, Sung P, Myung K. 2012. Rad5-dependent DNA repair functions of the *Saccharomyces cerevisiae* FANCM protein homolog Mph1. *J Biol Chem* 287: 26563–26575. <https://doi.org/10.1074/jbc.M112.369918>.
 57. Liefshitz B, Parket A, Maya R, Kupiec M. 1995. The role of DNA repair genes in recombination between repeated sequences in yeast. *Genetics* 140:1199–1211.
 58. James P, Halladay J, Craig EA. 1996. Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* 144:1425–1436.
 59. Liefshitz B, Steinlauf R, Friedl A, Eckardt-Schupp F, Kupiec M. 1998. Genetic interactions between mutants of the ‘error-prone’ repair group of *Saccharomyces cerevisiae* and their effect on recombination and mutagenesis. *Mutat Res* 407:135–145. [https://doi.org/10.1016/s0921-8777\(97\)00070-0](https://doi.org/10.1016/s0921-8777(97)00070-0).
 60. Friedl AA, Liefshitz B, Steinlauf R, Kupiec M. 2001. Deletion of the SRS2 gene suppresses elevated recombination and DNA damage sensitivity in rad5 and rad18 mutants of *Saccharomyces cerevisiae*. *Mutat Res* 486: 137–146. [https://doi.org/10.1016/s0921-8777\(01\)00086-6](https://doi.org/10.1016/s0921-8777(01)00086-6).